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Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods

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Moxalactam, nalidixic acid and bacitracin were found to be an effective combination of selective agents that permitted growth of Listeria *while suppressing most foodborne genera. These selective agents were used to formulate Modified Vogel Johnson Agar which permitted the quantitative detection of* Listeria *from foods.* Listeria *were characteristically tellurite$^+$/mannitol$^-$ and could be readily differentiated without the need to view the colonies with oblique reflected light. The combination of selective agents was also effective when employed in conjunction with Modified McBride Agar. Modified Vogel Johnson Agar proved highly effective for isolation of* Listeria *and should significantly enhance its detection in foods.*

Introduction

The recent identification of foods as an important potential cause of epidemic listeriosis outbreaks in humans has produced concern in regard to the adequacy of available methods for detecting and quantifying *Listeria monocytogenes* in various food products (Doyle and Schoeni 1987, Hao et al. 1987). Currently, Modified McBride Agar (Lovett et al. 1987) and LPM Agar (Lee and McClain 1986) are used routinely as plating media for the detection of *Listeria* from foods. However, it has been our experience that both media can suffer from lack of selectivity, particularly in regard to growth of co-contaminating staphylococci, streptococci and micrococci. Further, both media rely on subsequently detecting *Listeria* on the basis of colonies taking on a blue-gray color when illuminated with reflected light at a specific angle; a means of selection that is less than ideal. Hao et al. (1987) reported similar problems, concluding that these deficiencies prevented the use of direct plating as a means of detecting *Listeria* in foods. However, effective means of direct plating could greatly enhance monitoring foods for this psychrotrophic pathogen. Accordingly, the objective of the current study was to develop improved plating media that were quantitative, had improved selectivity, and did not rely on colonial illumination with reflected light. This objective was realized through the development of two media; an improved version of Modified McBride Agar and a new medium based on modifications of Vogel-Johnson Agar.

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Materials and Methods

Microorganisms

Studies into the development and subsequent characterization of the media routinely employed *Listeria monocytogenes* Scott A and *Staphylococcus aureus* 196E. Additional

species employed during the initial development of the media included *Listeria ivanovii* Kcl 114 type 5, *Listeria innocua* La-1 cheese NT, and *Micrococcus spp.* (hamburger isolate). Stock cultures were grown in Brain Heart Infusion (BHI) (Difco) for 24 h at 37°C and then stored at 4°C.

Table 1. Formulations for plating media developed for the detection of *Listeria*.

ARS-Modified McBride Agar (ARS-MMA)	
Phenylethanol Agar (Difco)	35·5 g
Lithium chloride	0·5 g
Glycine anhydride	10·0 g
Cycloheximide	0·2 g
Nalidixic acid	50mg
Moxalactam[a]	5 mg
Bacitracin[a]	20 mg
Distilled water	1000 ml
Modified Vogel Johnson Agar (MVJ)	
Vogel Johnson Agar (Difco)	60 g
Nalidixic acid	50 mg
Bacitracin[a]	20 mg
Moxalactam[a]	5 mg
1% Potassium tellurite soln[a]	20 ml
Distilled water	980 ml

[a] Bacitracin, moxalactam and potassium tellurite were added as filter sterilized stock solutions after medium was autoclaved for 15 min at 15 psi and then cooled to 50°C.

Media

Modified Vogel Johnson Agar (MVJ) and ARS-Modified McBride Agar (ARS-MMA), the media developed as a result of this study, are presented in Table 1. Various modifications to these basic formulations were evaluated, particularly in regard to optimizing antibiotic levels. These media were made in 1000 ml batches by initially preparing and autoclaving the heat stable components, tempering to 50°C, and then adding the filter-sterilized components (i.e. antibiotics and potassium tellurite). The media were then poured in 20 ml portions into culture dishes, allowed to solidify and stored in plastic bags at 4°C until used. Additional plating media used during the course of the investigations included Trypticase Soy Agar (Difco) and Modified McBride Agar (MMA) (Lovett et al. 1987). All media were used within 1 week of preparation.

Evaluation of selective agents

Sterile BHI (10 ml in 50-ml Erlenmeyer flasks) was inoculated with 0·1 ml of stock culture of *L. monocytogenes* or other test species. The flasks were incubated for 20 h at 37°C on a rotary shaker (130 rpm) to obtain approximately 1×10^9 cfu ml^{-1}. The cultures were diluted in 0·1% peptone water to 1×10^5 cfu/ml and then surface plated using a Spiral Plater onto test media with various levels of selective agents and onto appropriate control plates (MMA or TSA). All plates were incubated at 37°C for 72 h with counts being made after 24, 48 and 72 h.

Recovery studies

L. monocytogenes was grown as described above and diluted in 0·1% pepteone water to 1×10^5 cfu ml^{-1}. Retail samples (150 g) of milk (pasteurized, homogenized), sterile milk, hamburger, hamburger treated with-irradiation (4·2 Mrad), sausage, cheese (Brie), chicken salad, ice cream (chocolate, melted), and cole slaw were inoculated with 1·5 ml of the diluted culture and mixed thoroughly. The inoculated food samples were subdivided into six 25 g subsamples. Two uninoculated 25 g samples of each food were used as controls. Three inoculated and one uninoculated samples were placed in individual stomacher bags along with 100 ml of sterile 0·1% peptone water and processed for 1 min (milk and ice cream samples were not subjected to this step). Appropriate dilutions were made, surface plated in 0·1 ml portions onto MVJ, ARS-MMA and TSA. All plates were incubated at 37°C, with the MVJ and TSA plates being counted after 48 h and the ARS-MMA plates after 72 h. The remaining inoculated and uninoculated food samples were stored at 4°C and then analyzed in the same manner.

Results

Preliminary experimentation with Vogel Johnson Agar (VJ) (Difco) indicated that pure cultures of *L. monocytogenes* could be quantitatively recovered; characteristically the colonies were entirely black (tellurite-positive) on a red background (mannitol-negative). However, when recovery studies with retail foods were attempted there was significant interference due to co-

Table 2. Percent recovery of *Listeria monocytogenes* and *Staphylococcus aureus* when varying concentrations of moxalactam, nalidixic acid and bacitracin were added to Modified Vogel Johnson Agar and ARS-Modified McBride Agar.

Antibiotic	Conc. (mg l^{-1})	*L. monocytogenes*		*S. aureus*	
		MVJ	ARS-MMA	MVJ	ARS-MMA
None	0	103	102	99	103
Moxalactam	5	101	101	96	0
	10	102	100	0	0
	15	101	102	0	0
	20	100	101	0	0
	25	99	101	0	0
Bacitracin	5	101	101	96	103
	10	100	103	84	85
	15	98	102	79	132
	20	101	101	84	0
	25	102	99	80	41
Nalidixic acid	10	90	104	97	99
	20	101	102	99	41
	25	100	101	96	0
	50	104	101	0	0
Mox/Bac/Nal	5/20/50	100	102	0	0

contaminating micrococci, streptococci and staphylococci. *S. aureus* was particularly troublesome due to its strong mannitol-positive reaction masking mannitol-negative *Listeria* that were also on the plates. Further, it was resistant to many of the same selective agents as *L. monocytogenes*. Accordingly, subsequent experimentation concentrated on assessing selective agents, alone and in combination, that could be used in conjunction with VJ and MMA to suppress *S. aureus* without affecting the recovery of *L. monocytogenes*.

A combination of moxalactam, bacitracin and nalidixic acid was found to be very useful as a selective agent (Table 2). When these antibiotics were used at concentrations of 5, 20 and 50 mg l^{-1}, respectively, the growth of *S. aureus* and other microorganisms in food samples could be effectively controlled while still permitting the quantitative recovery of *L. monocytogenes*. This combination of antibiotics became a key component of the final formulations for MVJ and ARS-MMA (Table 1).

The recovery of *L. monocytogenes* from various foods was quantitative (Table 3). Recoveries were slightly reduced in meat samples, though still within the acceptable range for effective enumeration method. Subsequent studies (not shown) indicated that the reduced recovery rate in hamburger may have been due in part to an over estimation of the size of the inoculum, and that the actual recovery rate is in the 80–90% range. Recoveries tended to be higher in MVJ than ARS-MMA, though this varied among specific foods. These media could be used effectively to monitor the growth of *L. monocytogenes* in various refrigerated foods (Table 4). Interestingly, the strain of *L. monocytogenes* used (Scott A) grew in the dairy products but did not grow in the meat or poultry products. The commercial cole slaw used in the experiment had a pH of 3·8 and was lethal to *L. monocytogenes*. Additional studies (data

Table 3. Ability of ARS-MMA and MVJ to quantitatively recover *Listeria monocytogenes* from various foods.

Food	% Recovery[b]	
	MVJ	ARS-MMA
Milk, pasteurized	102 (7)	88 (10)
Milk, sterilized	118 (19)	101 (8)
Ice cream	107 (7)	85 (6)
Cheese, Brie	147 (79)	93 (33)
Hamburger	60 (18)	60 (19)
Hamburger, irradiated	80 (27)	96 (33)
Sausage	87 (7)	94 (20)
Chicken salad	111 (17)	104 (28)
Cole slaw	105 (12)	93 (16)

[a] Foods inoculated to contain approximately 10^3 cfu g^{-1}.
[b] Values represent mean (±SEM) where n = at least three determinations done in duplicate.

not shown) indicated that these media could be used for direct plating to monitor foods having as few as 10–20 cfu g^{-1}.

Discussion

The simultaneous use of moxalactam, bacitracin and nalidixic acid proved to be an effective combination of selective agents that supported the growth of *Listeria*, while suppressing the growth of other species, including *S. aureus*. The combination performed well in conjunction with both ARS-MMA and MVJ, permitting quantitative recoveries. Slightly higher recovery rates were achieved generally with MVJ. Conversely, ARS-MMA tended to be more selective. However, the performance of MVJ did not suffer due to this slightly reduced level of selectivity because the tellurite and mannitol reactions allowed ready differentiation of *Listeria* from other microorganisms that were resistant to the selective agents. It also should be noted that the antibiotic concentrations used in the current formulations (Table 1) were maintained at minimal levels to insure quantitative recovery of *Listeria*. The selectivity of both media can be increased by increasing the levels of antibiotics, particularly moxalactam.

ARS-MMA still required the detection of a blue-gray color upon illumination with reflected light to differentiate *Listeria* colonies. MVJ was designed to eliminate this approach to differentiation and for that reason was easier to use. Tellurite reduction was chosen as the alternate means of differentiating *Listeria* because this biochemical characteristic is visually distinctive and restricted to relatively few microorganisms. Further, tellurite is selective, inhibiting the growth of a range of Gram negative species. Tellurite has been used previously for the isolation of *Listeria* (Schoer 1944, Gray et al 1950), but did not gain wide acceptance, in part due to reports that it was inhibitory (Leighton 1979). However, our results with MVJ have indicated that though tellurite may reduce the rate of growth to a small degree, it does not interfere with the quantitative detection of the microorganism (Table 4). A possible exception to this is the detection of cells that have been injured due to sublethal thermal processing (Smith and Archer 1988). When employing MVJ, care should be taken in examining the extent of the tellurite reaction. *Listeria* characteristically form colonies that are entirely black. Colonies not entirely black have been consistently found not to be *Listeria* upon confirmation. For example, black (tellurite-positive), mannitol-negative colonies that have a white ring around their circumference typically have been found to be streptococci resistant to the selective agents.

Attempts to modify these media so that the different species of *Listeria* could be differentiated directly have so far been unsuccessful. For example, it was hoped that the incorporation of xylose into MVJ would permit *L. monocytogenes* and *L. innocua* to be

Table 4. Use of ARS-MMA and MVJ to monitor the growth of *Listeria monocytogenes* Scott A in various foods after inoculation and subsequent storage at 4°C for 7 days.

Food	Prior to inoculation			After inoculation			After 7 days at 4°C		
	Total mesophiles	*L. monocytogenes*		Total mesophiles	*L. monocytogenes*		Total mesophiles	*L. monocytogenes*	
		ARS-MMA	MVJ		ARS-MMA	MVJ		ARS-MMA	MVJ
Milk, pasteurized	3·14	N.D.[b]	N.D.	3·80	3·64	3·71	5·26	5·00	5·25
Milk, sterilized	N.D.	N.D.	N.D.	3·82	3·83	3·98	5·46	5·02	5·21
Ice cream mix	2·16	N.D.	N.D.	3·77	3·68	3·79	5·60	4·71	5·39
Cheese, Brie	9·43	N.D.	N.D.	9·40	3·10	3·28	9·45	4·13	5·18
Hamburger	7·84	N.D.	N.D.	7·88	3·35	3·36	8·81	3·30	3·38
Hamburger, irradiated	2·48	N.D.	N.D.	3·29	3·20	3·12	7·15	4·36	4·17
Sausage	5·88	N.D.	N.D.	5·84	3·59	3·58	7·32	3·38	3·50
Chicken salad	3·51	N.D.	N.D.	3·23	3·66	3·67	8·80	3·32	3·22
Cole slaw	4·57	N.D.	N.D.	4·70	3·59	3·65	5·05	N.D.	N.D.

[a] Foods inoculated to contain approximately 10^3 cfu g^{-1}.
[b] None detected.

distinguished from other *Listeria* species based on a mannitol-negative, xylose-negative response. Likewise, addition of rhamnose and a pH indicator to ARS-MMA was evaluated to see if this could be used to help differentiate *L. monocytogenes*. In both cases, *Listeria* species appeared to be insufficiently fermentative on these media to allow accurate direct speciation. Instead, suspect colonies had to be tested subsequently using Purple Broth plates or tubes containing the appropriate carbohydrates.

Ability to monitor levels of *L. monocytogenes* quantitatively allowed observation of the growth of the organism. One particularly interesting effect noted that warrants further investigation is differences in the growth of *L. monocytogenes* in different foods (Table 4). Dairy products held at 4°C consistently supported growth of *L. monocytogenes* whereas meat and poultry products supported survival but not growth of the microorganism. Outbreaks of epidemic human listeriosis have been traced in dairy products, but as yet have not been associated with meat or poultry products. Some growth was observed in hamburger treated with radiation, suggesting a possible competitive effect. Further studies are underway to confirm these observations. The lethality observed in the cole slaw samples was in agreement with the results of Beuchat et al. (1986) and Conner et al. (1986) who showed the minimum pH for growth of *Listeria* to be 4·2.

These two new media appear to offer substantial improvements over plating media available currently for the isolation of *Listeria*, particularly in regard to quantitatively monitoring the microorganism. MVJ appears particularly promising due to its ability to readily differentiate *Listeria* from other microorganisms, and should permit detection by direct plating even when *Listeria* is present in samples at relatively low numbers. Further, in our laboratory MVJ has been proven itself to be extremely useful when used in conjunction with most probable number preenrichment schemes similar to that described by Lee and McClain (1986). The effectiveness of both media for detecting *Listeria* in a variety of retail foods is being assessed and will be reported separately.

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